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Original communication

Two new oro-cervical radiographic indexes for chronological age estimation: A pilot study on an Italian population

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ABSTRACT

Chronological age (CA) plays a fundamental role in forensic dentistry (i.e. personal identification and evaluation of imputability). Even though several studies outlined the association between biological and chronological age, there is still great variability in the estimates. The aim of this study was to determine the possible correlation between biological and CA age through the use of two new radiographic indexes (Oro-Cervical Radiographic Simplified Score – OCRSS and Oro-Cervical Radiographic Simplified Score Without Wisdom Teeth – OCRSSWWT) that are based on the oro-cervical area. Sixty Italian Caucasian individuals were divided into 3 groups according to their CA: Group 1: CAG 1 = 8–14 yr; Group 2: CAG 2 = 14–18 yr; Group 3: CAG 3 = 18–25 yr; panorexes and standardised cephalograms were evaluated according Demirjian's Method for dental age calculation (DM), Cervical Vertebral Maturation method for skeletal age calculation (CVMS) and Third Molar Development for age estimation (TMD). The stages of each method were simplified in order to generate OCRSS, which summarized the simplified scores of the three methods, and OCRSSWWT, which summarized the simplified DM and CVMS scores. There was a significant correlation between OCRSS and CAGs (Slope = 0.954, $p < 0.001$, R-squared = 0.79) and between OCRSSWWT and CAGs (Slope = 0.863, $p < 0.001$, R-squared = 0.776). Even though the indexes, especially OCRSS, appear to be highly reliable, growth variability among individuals can deeply influence the anatomical changes from childhood to adulthood. A multi-disciplinary approach that considers many different biomarkers could help make radiological age determination more reliable when it is used to predict CA.

1. Introduction

Chronological age (CA) plays a fundamental role in forensic dentistry. The evaluation of imputability (i.e., defendants or illegal immigrants without identification documents) is the most important emerging fields in which CA determination can have a large impact.[1–3] During legal trials in which a supposed minor who does not possess identifying documents is involved, the court needs to ascertain the defendant's exact CA to determine imputability. The court usually requests an expert, such as a forensic doctor or an auxologist, to establish the CA of the defendant. The methods routinely used to determine CA are mainly based on the determination of biological age (i.e., dental or bone age), which, unfortunately, can only predict CA. Even though several studies outlined the association between biological and chronological age, and some notable recommendations have been published, there is still great variability in the estimates.[4–11]

Italian law considers CA to be an essential requirement for imputability. To this end, three major CA groups are classified as follows[12]:

- individuals younger than 14 years are not imputable;
- for individuals between 14 and 18 years, the imputability must be established on a case-by-case basis through psychiatric evaluation *(doli incapax test)*;
- individuals older than 18 years are imputable, unless there are pathological circumstances that impair mental and physical integrity.

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Furthermore, Italian law provides a 24-hour period to determine CA. Because the defendant is considered to be the CA at which one receives the more lenient punishment, it is absolutely necessary to perform all identification procedures in the shortest amount of time.[12]

Determining the CA through the assessment of biological age using radiographic growth parameters (i.e., the hand and wrist skeletal development atlas[6] and Demirjian's method for dental age calculation[5]) is the most widely used method. The growth of the stomatognatic region has been frequently advocated as a precise marker of biological age, particularly from birth to puberty (12–14 years old). Tooth development was initially thought to be slightly influenced by the environment and diseases; nevertheless, great differences in tooth development were observed among individuals of the same age living in the same country but in different regions.[13–15] Furthermore, after puberty, age determination can be only performed by considering third molar development, which is associated with substantial variance (i.e., absence, malformed and high variability for the degree of maturation).[16–21]

Even though radiographic methods are used most frequently, many studies have failed to determine a precise correlation between biological age and CA. Moreover, these methods define an age range of the defendant; therefore, the practical application according to Italian law implies that the defendant could be assigned to two different imputability-CA groups, and may thus be eligible for two different legal procedures. For instance, a defendant could be identified as being between 17 and 19 years old. In this situation, Italian law considers the defendant to be the CA at which the more lenient sentence is administered.[1–3,7–9,22–24]

The aim of this study was to determine the possible correlation between biological age and CA through the use of two new radiographic indexes; these are based on the combination of already validated biological age calculation methods performed on the oro-cervical area in an Italian population with the purpose of determining the imputability of supposed minors without age identifying documents. The first method, called Oro-Cervical Radiographic Simplified Score (OCRSS), is based on the combination and simplification of three already established radiographic indexes:

A. Demirjian's Method for dental age calculation (DM)[5]
B. Cervical Vertebral Maturation method for skeletal age calculation (CVMS)[25]
C. Third Molar Development for age estimation (TMD)[26]

The second method, referred to as Oro-Cervical Radiographic Simplified Score Without Wisdom Teeth (OCRSSWWT), is derived from the combination and simplification of only two of the above mentioned indexes: DM and CVMS.

The OCRSS index assessed the accuracy of the correlation between this new oro-cervical index and CA under the best radiographic and anatomical conditions; the OCRSSWWT index assessed the accuracy of the correlation when only two oro-cervical biological age indexes were available (when the third molar was missing) and the possible contribution of CVMS when TMD could not be used.

2. Materials and methods

Sixty Italian Caucasian individuals (21 males, 39 females; Table 1), all belonging to middle class socioeconomic status, were retrospectively enrolled among orthodontic outpatients of the Dental Clinic (Catholic University, Rome), in two years study (2010–2012). The patients were divided into 3 groups according to

Table 1
Characteristics of the population stratified on CA, OCRSS and OCRSSWWT.

		N°	Mean	Std. deviation	Minimum	Maximum	95% CI for mean	
							Lower bound	Upper bound
CAG 3	Total	20	21.24	2.35	18	25	20.6	23.3
	F	11	20.26	1.97	18	23.6	19.1	22.8
	M	9	22.44	2.37	19.6	25	20.9	25.1
CAG 2		20	15.62	1.37	14	17.7	15.4	17.2
	F	16	15.66	1.41	14	17.7	15.2	17.1
	M	4	15.50	1.39	14	17.3	14.2	19.9
CAG 1	Total	20	10.38	1.46	8.5	13.3	9.6	10.9
	F	12	9.78	1.15	8.5	12.5	9	10
	M	8	11.29	1.46	9.1	13.3	10.1	12.5
OCRSS C[a]	Total	14	21.99	2.36	18.1	25	20.6	23.3
	F	7	20.91	2.05	18.1	23.6	19	22.8
	M	7	23.05	2.26	19.6	25	20.9	25.1
OCRSS B[a]	Total	27	16.37	2.22	12.5	21	15.5	17.2
	F	21	16.16	2.09	12.5	21	15.2	17.1
	M	6	17.1	2.71	14	20.7	14.2	19.9
OCRSS A[a]	Total	19	10.27	1.41	8.5	13.3	9.6	10.9
	F	11	9.53	0.80	8.5	11.2	8.9	10
	M	8	11.28	1.46	9.1	13.3	10.1	12.5
OCRSSWWT C[b]	Total	19	20.67	3.19	14.8	25	19.1	22.2
	F	11	19.22	2.98	14.8	23.6	17.2	21.2
	M	8	22.66	2.37	19.6	25	20.7	24.6
OCRSSWWT B[b]	Total	18	16.64	2.21	14	21	15.5	17.7
	F	14	16.56	2.12	14	21	15.3	17.8
	M	4	16.92	2.85	14	20.7	12.9	21.5
OCRSSWWT A[b]	Total	23	10.99	2.11	8.5	16	10.1	11.9
	F	14	10.52	2.21	8.5	16	9.2	11.8
	M	9	11.7	1.85	9.1	15	10.3	13.1

Linear regression model is reported in Results section.
[a] Differences between groups for mean age: ANOVA = $p < 0.001$ – Differences between each sub-group: Bonferroni Test = $p < 0.001$.
[b] Differences between groups for mean age: ANOVA = $p < 0.001$ – Differences between each sub-group: Bonferroni Test = $p < 0.001$.

their chronological age: Group 1: CAG 1 = 8–14 yr; Group 2: CAG 2 = 14–18 yr; Group 3: CAG 3 = 18–25 yr. One researcher, with at least 3 years of orthodontic experience, blindly evaluated panorexes and standardised cephalograms that were previously performed for orthodontic reasons; therefore, no further radiograms were performed. The inclusion criteria for the study were healthy individuals, no cranio-facial abnormalities, molar class I, good quality radiograms (panorex and cephalogram), and the presence of all lower left mandible teeth (third molar included).

All radiograms were all required for orthodontic purposes and were evaluated according to three different previously established radiographic indexes (Fig. 1): DM[5], CVMS[25] and TMD.[26] The stage of each method was simplified as follows: DM stages A–G: 0 points and stage H: 1 point; CVMS stages I–III: 0 points, stage IV: 1 point and stages V and VI: 2 points; TMD for age estimation stages A and B: 0 points, stages C–F: 1 point and stages G and H: 2 points. The addition of the simplified scores generated two new oro-cervical radiographic indexes. First, the OCRSS was the result of the addition of the simplified scores of the three methods. Second, the OCRSSWWT was the result of the addition of the simplified DM and CVMS scores. Based on the new indexes, the population was divided into 3 groups that should be representative of increasing CA (Fig. 1):

OCRSS:
- A. Sum of simplified scores 0 or 1: OCRSS A;
- B. Sum of simplified scores 2–4: OCRSS B;
- C. Sum of simplified scores 5: OCRSS C;

OCRSSWWT:
- A. Sum of simplified scores 0–1: OCRSSWWT A;
- B. Sum of simplified scores 2: OCRSSWWT B;
- C. Sum of simplified scores 3: OCRSSWWT C;

Thus, it was possible to correlate the new indexes with the CAGs and CA.

2.1. Statistical analyses

Quantitative variables were tested for normal distribution using a Shapiro–Wilk test. Parametric variables were compared using a two-tailed ANOVA. If significance was determined, a *post hoc* Bonferroni test was performed to define the differences between the groups. A linear regression model was used to calculate the possible correlation between OCRSS or OCRSSWWT and CAGs or CA. Frequency variables were assessed using the Chi-Squared test and Fisher's exact test. A *p*-value of $p \leq 0.05$ was considered to be statistically significant. The statistical analyses were performed using the Intercooled Stata 8.0 software (Stata Corporation, College Station, TX, USA).

3. Results

Characteristics of the population are reported in Table 1. The stratified population based on OCRSS and OCRSSWWT is also reported in Table 1.

3.1. Oro-Cervical Radiographic Simplified Score (OCRSS)

Nineteen individuals were assigned to group OCRSS A, 27 to group OCRSS B and 14 to OCRSS C. The differences among the mean age of the three groups were statistically significant even when the Bonferroni test was applied ($p < 0.001$). There was a significant correlation between the OCRSS groups and CA (Slope = 5.44, $p < 0.001$, R-squared = 0.75), and between the OCRSS groups and CAGs (Slope = 0.954, $p < 0.001$, R-squared = 0.79). The correlation remained significant when the sample was stratified by sex. For females, there was a significant correlation between the OCRSS groups and CA (Slope = 5.32, $p < 0.001$, R-squared = 0.78), as well as between the OCRSS groups and CAGs (Slope = 0.952, $p < 0.001$, R-squared = 0.78). For males, there was a significant correlation between the OCRSS groups and CA (Slope = 5.81, $p < 0.001$, R-squared = 0.81), as well as between the OCRSS groups and CAGs (Slope = 0.971, $p < 0.001$, R-squared = 0.9). The distribution of individuals based on the CAG and OCRSS groups is reported in Table 2, and the differences among the groups were statistically significant.

3.2. Oro-Cervical Radiographic Simplified Score Without Wisdom Teeth (OCRSSWWT)

Twenty-three individuals were assigned to group OCRSSWWT A, 18 individuals to group OCRSSWWT B and 19 individuals to OCRSSWWT C. Differences among the mean age of the three groups

Biological age method	Stage		Simplified score
Demirjian's Method	A to G	➔	0
	H	➔	1
Cervical Vertebral Maturation method for skeletal age calculation	I to III	➔	0
	IV	➔	1
	V and VI	➔	2
Third Molar Development for age estimation	A and B	➔	0
	C to F	➔	1
	G and H	➔	2

Oro-Cervical Radiographic Simplified Score (OCRSS)	Addition of simplified scores
OCRSS A	0-1
OCRSS B	2-4
OCRSS C	5

Oro-Cervical Radiographic Simplified Score Without Wisdom Teeth (OCRSSWWT)	Addition of simplified scores
OCRSSWWT A	0-1
OCRSSWWT B	2
OCRSSWWT C	3

Fig. 1. The figure shows Demirjian's Method for dental age calculation (DM)[5], Cervical Vertebral Maturation method for skeletal age calculation (CVMS)[25], Third Molar Development for age estimation (TMD)[26] scores and the method used to obtain the simplified scores.

Table 2
The distribution of participants based on the CAG, OCRSS and OCRSSWWT groups.

			OCRSS			
			A	B	C	Total
CAG[a]	1	<14	19	1	0	20
	2	14–18	0	20	0	20
	3	>18	0	6	14	20
		Total	19	27	14	60
CAG − FEMALES	1	<14	11	1	0	12
	2	14–18	0	15	1	16
	3	>18	0	3	8	11
		Total	11	19	9	39
CAG − MALES	1	<14	8	0	0	8
	2	14–18	0	4	0	4
	3	>18	0	2	7	9
		Total	8	6	7	21
			OCRSSWWT			
			A	B	C	Total
CAG[a]	1	<14	20	0	0	20
	2	14–18	3	14	3	20
	3	>18	0	4	16	20
		Total	23	18	19	60
CAG − FEMALES	1	<14	12	0	0	12
	2	14–18	2	11	3	16
	3	>18	0	3	8	11
		Total	14	14	11	39
CAG −MALES	1	<14	8	0	0	8
	2	14–18	1	3	0	4
	3	>18	0	1	8	9
		Total	9	4	8	21

[a] χ^2 Fisher's exact test $p < 0.001$.

were statistically significant even when the Bonferroni test was applied.

There was a significant correlation between the OCRSSWWT groups and CA (Slope = 4.867, $p < 0.001$, R-squared = 0.721), as well as between the OCRSSWWT groups and CAGs (Slope = 0.863, $p < 0.001$, R-squared = 0.776). The correlation remained significant when the sample was stratified by sex. For females, there was a significant correlation between OCRSSWWT groups and CA (Slope = 4.423, $p < 0.001$, R-squared = 0.672), as well as between the OCRSSWWT groups and CAGs (Slope = 0.804, $p < 0.001$, R-squared = 0.698). For males, there was a significant correlation between the OCRSSWWT groups and CA (Slope = 5.478, $p < 0.001$, R-squared = 0.848), as well as between the OCRSSWWT groups and CAGs (Slope = 0.947, $p < 0.001$, R-squared = 0.896). The distribution of individuals according to CAG and OCRSSWWT is reported in Table 2, and the differences among the groups were statistically significant.

4. Discussion

The aim of this study was to evaluate an easy, fast and reliable tool for CA determination, particularly to determine the imputability of an individual who possesses no age-identifying documents or the age of asylum-seeking immigrants. The need for such a tool is crucial because in many European Union Countries (EU), and particularly in the Mediterranean Area (i.e., Italy, Malta, Greece, and Spain), illegal immigration fluxes are a major concern. Due to its key geographical position, Italy is a preferred location for illegal immigrants.[2]

It is well known that the most renowned procedures used to assess biological age are the hand and wrist skeletal development atlas[6] and the Cervical Vertebral Maturation method for skeletal age calculation.[25] These techniques, which are mainly used for orthodontic purposes, have been applied in the forensic field to determine CA; however, the expert reports frequently do not fulfil the Court's requests even when sophisticated computer aided methods are used. Indeed, an estimated CA may vary within a wide range, even greater than one year.[1,7,22,24] This uncertainty may shift the legal position of the defendant from one age group to another and thus substantially interfere with the determination of imputability.[12]

We evaluated 60 Italian (Caucasian) individuals for the correlation between CA and two new oro-cervical radiographic indexes (OCRSS and OCRSSWWT) that were derived from the combination of the already well-established craniofacial radiological methods (DM[5], TMD[26] and CVMS[25]). The first score, OCRSS, assessed the accuracy of the correlation between a new oro-cervical index that combines all 3 oro-cervical radiographic indexes and CA at the best radiographic and anatomical conditions. The second one, OCRSSWWT, assessed the accuracy of the correlation when TMD could not be used (i.e., when the third molars were missing).

The most important result of this study is the highly statistically significant correlation between OCRSS and CA (Slope = 5.44, $p < 0.001$, R-squared = 0.75), and the result between the OCRSS groups and CAGs (Slope = 0.954, $p < 0.001$, R-squared = 0.79). Considering the distribution of the single individuals into the CAGs according to the OCRSS index (Table 2), further conclusions can be drawn, especially based on the reliability of the OCRSS index: just one subject from CAG 1 (8–14 years old) was identified as belonging to the OCRSS B group, which should indicate an older age for that individual. Therefore, in the lower age group, OCRSS can be considered highly reliable. This is likely due to the information carried into this model by DM, which has good consistency in younger individuals. It is interesting to note that even if the correlation remained statistically significant when sample was stratified by sex, the individual incorrectly assigned to OCRSS B was female. One possible explanation is that hormonal factors could have resulted in earlier maturation leading to this overestimation.[27]

Further observations of OCRSS B can be outlined (Table 2). Six individuals from CAG 3 were incorrectly assigned to group OCRSS B; thus, the OCRSS index appears to underestimate age in the oldest group. This result provides some important and practical implications. For example, in a legal trial, the OCRSS index could be in favour of the defendant according to *in dubio pro reo*.[12] Moreover, it is worth noting that no individuals in the GAG B group were incorrectly assigned to the OCRSS C group.

The results from the OCRSSWWT index highlight the information from the use of CMV when TMD cannot be used (third molars absent, anomalous shape or with a clearly delayed maturation time). It is well-known that DM can produce reliable and precise information in individuals up to 14 years old; whereas, for individuals older than 14 years, the third molars remain the only anatomical structure that can provide information for age calculation in a panorex (14–16).

The OCRSSWWT index (Table 2) was significantly correlated with CA (Slope = 4.867, $p < 0.001$, R-squared = 0.721) and CAG (Slope = 0.863, $p < 0.001$, R-squared = 0.776) even though this index exhibited a lower performance than OCRSS.

Based on the distribution of the individuals in the CAGs according to the OCRSSWWT index (Table 2), some conclusions can be drawn about its reliability. In the first group (OCRSSWWT A), all 20 CAG 1 individuals were identified correctly, thus suggesting strong reliability when analysing younger individuals. However, 3 CGA B individuals were incorrectly assigned to OCRSSWWT A. Considering the correlation between CAG 2 and OCRSSWWT B and the correlation between CAG 3 and OCRSSWWT C, the ages of the 3 individuals from CAG 2 were overestimated, and they were

identified as majors (all were females); thus, this index seems to overestimate the age of the individuals. Therefore, when the OCRSSWWT index is applied to assess CA in younger individuals, it appears to be in favour of the defendant; however, in older individuals, it is not beneficial for the defendant.

Taken together, even though the indexes, especially OCRSS, appear to be reliable, fast and convenient (both panorex and cephalograms can be performed using the same radiological appliance at a low x-ray dose), an underestimation of the chronological age in OCRSS C and an overestimation of the chronological age in OCRSSWWT C must be taken into consideration. The growth variability among individuals can deeply influence the anatomical changes from childhood to adulthood.[27] We agree with the study recently published by Thevissen et al.: a multi-disciplinary approach that takes into consideration many different biomarkers (i.e., anthropometric, psychometric, hormonal and genomic) could help make radiological age determination more reliable when it is used to predict CA.[28] It is our opinion that imputability is highly related to brain maturity and biological age more than CA, which cannot always be determined in a precise and certain manner.

Furthermore, as can be seen in Table 3, great differences can be noted on the minim age of criminal responsibility among different Countries: in the light of complex migratory fluxes and of a incessant globalization process, it is absolutely mandatory to settle worldwide the relationship between age and imputability.[29–32]

Table 3
Criminal responsibility among different Countries.

Country	MACR	*Doli incapax test*
Austria	14	
Belgium	18	
Bulgaria	14	14–18
Czech Republic	15	
Denmark	15	
England	10	
Estonia	14	
Finland	15	
France	13	
Germany	14	14–18
Greece	13	
Hungary	14	
Ireland	10	
Italy	14	14–18
Latvia	14	
Lithuania	14[a]/16	
Norway	15	
Netherlands	12	
Poland	15	
Romania	14	14–16
Russia	14[a]/16	
Scotland	12	
Slovakia	14	14–15
Slovenia	14[a]/16	
Spain	14	
Sweden	15	
Switzerland	10	
Turkey	12	
Ukraine	14[a]/16	
United States of America		
NC	6	
NY, MS, MA	7	
AR, WA	8	8–12 WA
CO, KA, LO, MI, PE, TX	10	

MACR = The minimum age of criminal responsibility.
AR = Arizona; CO = Colorado; KA = Kansas; LO = Louisiana; MA = Maryland; MI = Minnesota; MS = Massachusetts; NC = North Carolina; NY = New York; PE = Pennsylvania; TX = Texas; WA = Washington.
[a] Only for serious offences.

4.1. Limitations of the study

Some limitations of this pilot study should be highlighted. First, this work has been performed only using Italian Caucasians. Therefore, further studies are necessary to confirm the reliability of these indexes when they are applied to individuals of different races. The study performed by Lewis et al.[16] concluded that age determination within individuals from the same Country can be highly variable up to 14 months of age, mainly due to racial differences. Moreover, some other important variables (i.e., nutritional differences and other radiological findings such as clavicular ossification[33]) can contribute to high variability and should be taken into consideration. Second, even if these new indexes appear to be reliable, this study was performed on a small number of individuals using the best radiological conditions; thus, indexes should be validated with a larger sample size, and possible differences due to different radiological appliances should be evaluated. Finally, intra-observer and inter-observer agreement should be further evaluated to strengthen the reliability of the indexes.

5. Conclusions

The new indexes, especially OCRSS, have proven to be easy, fast and reliable tools for CA determination, particularly to determine the age of individuals who possess no age-identifying documents or the age of asylum-seeking immigrants, nevertheless a multi-disciplinary approach that considers many different biomarkers could help make radiological age determination more reliable when it is used to predict CA.

Ethical approval
Not needed.

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Conflict of interest
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